Females manipulate behavior of caring males via prenatal maternal effects

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Abstract:

In species with biparental care, there is sexual conflict as each parent is under selection to minimize its personal effort by shifting as much as possible of the workload over to the other parent. Most theoretical and empirical work on the resolution of this conflict has focused on strategies used by both parents, such as negotiation. However, because females produce the eggs, this might afford females with an ability to manipulate male behavior via maternal effects that alter offspring phenotypes. To test this hypothesis, we manipulated the prenatal conditions (i.e., presence or absence of the male), performed a cross-fostering experiment, and monitored subsequent effects of prenatal conditions on offspring and parental performances in the burying beetle *Nicrophorus vespilloides*. We found that offspring were smaller at hatching when females laid eggs in presence of a male, suggesting that females invest less in eggs when expecting male assistance. Furthermore, broods laid in the presence of a male gained more weight during parental care, and they did so at the expense of male weight gain. Contrary to our expectations, males cared less for broods laid in the presence of a male. Our results provide experimental evidence that females can alter male behavior during breeding by adjusting maternal effects according to prenatal conditions. However, rather than increasing the male's parental effort, females appeared to suppress the male's food consumption, thereby leaving more food for their brood.

Significance

In biparental species, sexual conflict arises as each parent attempts to minimize its personal effort. Most work has focused on how this conflict is resolved through symmetrical decisions between parents. We investigated whether females can influence male decisions by altering the offspring's phenotype via the eggs. We manipulated the prenatal presence of the male, performed a cross-fostering experiment, and monitored the subsequent effects on offspring and parent performances. Offspring laid in presence of a male were smaller at hatching. Additionally, males lost more weight when with larvae laid in presence of a male. Our results show that females can manipulate male behavior, suggesting that prenatal maternal effects can play an important role in the resolution of sexual conflict between parents. *body*

Introduction

In species where parents cooperate to care for their joint offspring (as long as there is scope for divorce and/or re-mating following the partner's death), there will be sexual conflict over parental care with each parent being under selection to minimize its own effort and shift as much as possible of the workload to its partner (1). Previous empirical and theoretical work has focused mainly on three behavioral mechanisms that may mediate the resolution of this conflict (1, 2). First, incomplete compensation occurs when each parent increases its level of care in response to a reduction in its partner's contribution, but such that it does not fully correspond to its partner's reduction (3). Second, matching occurs when each parent adjusts its level of care to its partner's contribution by matching any increase or reduction in its partner's contribution in the same direction as its partner (4). Third, sealed bids models assume that each parent makes an initial fixed decision about how much care to provide irrespective of its partner's decision (5). There is some support for all three mechanisms from experimental studies on birds and other taxa (e.g. negociation: 6, matching: 7, sealed bid: 8). However, a meta-analysis of mate removal and handicapping experiments on birds found overall support for negotiation (2).

Our current understanding of the resolution of sexual conflict suggest that males and females employ the same behavioral strategies for resolving conflict (e.g., negotiation) (9). However, given that females produce the eggs, they might use their control over egg production as a mechanism for biasing conflict resolution in their favor. In many species, females deposit hormones and/or nutrients into the eggs that alter the offspring's behavior or development (9-11), thereby providing a potential tool for manipulating the behavior of caring males. For example, by

producing smaller eggs, females could redirect the costs of parental care from the prenatal period where they pay the full costs of egg production towards the postnatal period where the costs of rearing young are shared with the male (9). Alternatively, females may deposit yolk androgens that modulate offspring begging behavior in a way that alters the male's perception of offspring need, thereby increasing male contributions towards care (9-11). Thus, if prenatal maternal effects influence the resolution of sexual conflict over care, this would introduce an asymmetry of power between the two sexes with the female gaining the upper hand.

Currently, we lack conclusive evidence as to whether females can use prenatal maternal effects to manipulate the behavior of caring males (12-16). A main reason for this is that it is difficult to demonstrate female manipulation due to several challenges. First, females could manipulate males via several mechanisms, including yolk androgens, other egg components, egg size and egg coloration (4). Thus, existing experimental designs that focus on specific mechanisms risk targeting the wrong mechanism. Second, to demonstrate that prenatal maternal effects alter male behavior, it is essential to separate the effects of prenatal conditions from those of postnatal conditions. Third, there may be a limited time window for maternal manipulation because maternal effects on offspring behavior often wane as offspring develop (17-19). Fourth, in addition to sexual conflict over parental care, there may be sexual conflict over food consumption from shared resources (20, 21). Finally, to demonstrate female manipulation, it is crucial to document fitness benefits to females and/or offspring and fitness costs to males (4).

Here we report an experiment on the burying beetle *Nicrophorus vespilloides*, an insect exhibiting facultative biparental care (22). This species is ideal for studying

93 female manipulation of male behavior because it allows us to address all five
94 challenges listed above. First, we use a new experimental approach where we
95 target prenatal environmental conditions expected to influence female decisions on
96 maternal effects and then monitor downstream consequences for offspring size at
97 hatching, male and female care, and male, female and offspring fitness (9). Here,
98 we target a prenatal environmental condition that is essential for female
99 manipulation of males: the presence or absence of the male during egg laying.
100 Second, we conduct a cross-fostering experiment to separate pre- and postnatal
101 effects by giving each pair of beetles an experimental brood of newly hatched larvae
102 derived from one of our two treatment groups. In one treatment, larvae derived from
103 eggs laid when the male was present during egg laying, while in the other
104 treatment, larvae derived from eggs laid when the male was absent during egg
105 laying. Third, we recorded parental behaviors over 3 consecutive days, covering the
106 full duration of parental care from hatching until nutritional independence (23).
107 Fourth, we investigate sexual conflict over both parental care and food consumption
108 from a resource shared by both parents and their offspring (i.e., a small vertebrate
109 carcass). To this end, we recorded the body mass of males and females before and
110 after breeding. Finally, we monitored subsequent effects on post-breeding survival
111 of males and females as well as offspring growth and survival across different life
112 stages.
113
114 **Results**
115 We find evidence for prenatal maternal effects due to the presence or absence of
116 the male during laying on offspring size at hatching. Females that laid eggs in the
117 presence of a male partner produced larvae that were 3.4% lighter at hatching than

females that did so in the absence of a male (Fig. 1) (Estimate±SE = 1.85±0.826, $F_{1,59}$ = 5.02, P = 0.0289; table S1). There was no difference in the number of eggs laid by females in the presence or absence of a male partner ($F_{1,142}$ = 0.431, P = 0.512). Thus, our result provides evidence for an anticipatory maternal effect, whereby females reduce their prenatal investment in offspring when anticipating help from a male partner (24, 25).

We find evidence that females use maternal effects to manipulate the behavior of caring males, but maternal effects influenced male food consumption rather than male care. There was no evidence that males increased the amount of care they provided in response to prenatal maternal effects. Males did not adjust their direct care 1h after hatching (Z = 0.0716, P = 0.943; table S3), but in contrast to what we predicted, males provided *less* direct care 25h after hatching when caring for a foster brood derived from eggs laid in the presence of a male (Fig. 2A) (Estimate±SE = 1.02±0.477, Z = 2.15, P = 0.0319; table S3). We found that males gained less or lost more weight when caring for a foster brood derived from eggs laid in the presence of a male (Fig. 2B) (Estimate±SE = 0.0127±6.02e^{-04}, $F_{1,57}$ = 4.44, P = 0.0394, table S1). Males that cared more 25h after hatching also gained more weight suggesting that spending more time caring for larvae provided them with better access to the food resource (Fig. 2C) (lm, Estimate±SE = 1.58e^{-3}±6.72e^{-4},$F_{1,58}$ = 5.53, P = 0.0222). Finally, male weight gain decreased as the initial brood weight increased (Fig. 2D) (Estimate±SE = -2.67±0.925,$F_{1,57}$ = 8.32, P = 0.00552; table S1), indicating that males also adjust the amount of carrion they eat based on the larvae's size. Our findings suggest that maternal effects provide females with a means to manipulate the behavior of caring males by suppressing male food consumption from the shared resource.

We find no evidence that maternal effects influenced the behavior of caring females. There were no effects of prenatal maternal effect on female direct care 25h (Fig. 2A) or 49h after hatching (respectively Z = 0.626, P = 0.531, Z = -0.365, P = 0.715; table S3). As predicted under the hypothesis of female manipulation, females tended to care less 1h after hatching for larvae laid in presence of a male but this effect was marginally non-significant (Z = 1.93, P = 0.0539; table S3). In contrast to what we found for males, prenatal maternal effects did not affect female weight gain (Fig. 2B) ($F_{1,58}$ = 0.437, P = 0.511 table S1). Thus, these results confirm that the prenatal maternal effect affected male behavior, but had no impact on the female's own behavior.

We find evidence of immediate fitness consequences of prenatal maternal effects mediated through the change in male behavior. Foster broods derived from eggs laid in the presence of a male were heavier at dispersal (i.e., the end of the parental care period) than foster broods derived from eggs laid in the absence of a male (Fig.3) (Estimate±SE = -0.333±0.144, $F_{1,57}$ = 5.35, P = 0.0243; table S1). There were no significant effects of prenatal maternal effects on either the number of dispersing larvae or average larval weight (respectively $F_{1,58}$ = 2.04, P = 0.158; $F_{1,57}$ = 2.69, P = 0.106, table S1), suggesting that the greater brood weight when eggs were laid in the absence of a male was due to a relatively small increase in both number of offspring and average larval weight. We also found that broods that were heavier at dispersal were heavier at hatching (Fig. 3) (Estimate±SE = 50.3±22.1, $F_{1,57}$ = 5.16, P = 0.0269, table S1). Keeping in mind that foster broods derived from eggs laid in the presence of a male were lighter at hatching, this suggests that the maternal effects on postnatal brood weight were strong enough to override the initial differences in weight at hatching, which were in the opposite

direction from those at dispersal. We then added male weight gain to the model on brood mass to examine whether the maternal effects on male weight change explained why broods derived from eggs laid in the presence of a male were heavier at dispersal. We found that male weight change had a highly significant effect on brood weight (Estimate±SE = -9.93±2.81, $F_{1,58}$ = 12.5, P = 0.000810; table S4), and when we included male weight change, the effect of treatment was no longer significant ($F_{1,57}$ = 1.62, P = 0.208; table S4). There was also a significant effect of female weight change on brood weight (Estimate±SE = -7.58±2.75, $F_{1,56}$ = 7.62, P = 0.00779), but in contrast to what we found for males, the effect of treatment was still significant when female weight gain was added (Estimate±SE = -0.306±0.138, $F_{1,56}$ = 5.01, P = 0.0291;table S4). Thus, our results suggest that the beneficial effects of prenatal maternal effects on offspring growth were mediated through the reduction in male food consumption from the shared resource.

Finally, we examined long-term fitness consequences of prenatal maternal effects by looking at offspring survival until eclosion as an adult, adult size as well as their lifespan post eclosion. There was a nonsignificant trend for offspring laid in the presence of a male to survive better from dispersal to eclosion (lm, eclosion rate: $F_{1,57}$ = 3.80, P = 0.0561), and offspring laid in absence of a male lived longer as adults (Hazard coefficient±SE = -0.632±0.250, χ^2 = 6.39, P = 0.0114, table S2, Fig.S1). The longer adult lifespan of offspring laid in the absence of a male may be caused by selective disappearance if weaker offspring have higher mortality in the period before eclosion. There was a nonsignificant trend for males (but not females) to die sooner after caring for larvae derived from eggs laid in the presence of a male ($Z_{1,57}$ = 1.83, P = 0.0670,table S2, Fig. S2).

Discussion

Our results provide experimental evidence that females can manipulate the behavior of caring males through prenatal maternal effects and that females appear to suppress male food consumption from a resource that is shared by the two parents and their offspring. We found that females respond to male presence during laying by producing larvae that are lighter at hatching, suggesting that females redirect the costs of parental care from the prenatal period where they pay the full costs of egg production towards the postnatal period where the costs of care are shared with the male. We also found that males gained less weight when caring for broods derived from eggs laid in the presence of a male, that such broods gained more weight during parental care, and that the greater weight gain of these broods came at the expense of the male's weight gain. These findings suggest that the female's suppression of the male's food consumption was beneficial to the offspring as it enhanced their access to the shared resource. Our results indicate that female manipulation of male behavior was targeted towards sexual conflict over food consumption rather than conflict over parental care.

Previous work on sexual conflict between caring parents has mainly focused conflict over parental care (1, 2). Thus, our results suggest that sexual conflict over food consumption from shared resources may be more important than traditionally recognized. This suggestion is also consistent with theoretical predictions and prior empirical work on *Nicrophorus vespilloides*. Although explicit models of maternal effects as a tool for female manipulation of male behavior are lacking, they could be interpreted as a form of Stackleberg games where one parent (here the female) makes the initial decision about how much to contribute and the second parent consequently responds by deciding its contribution (26). In such game theoretical

models, the individual making the initial decision is expected to gain the upper hand (26). Thus, if sexual conflict is primarily over care, we should expect females to provide less care than males. This prediction is not supported in our system where females provide more care than males (27, 28). However, if sexual conflict occurs over food consumption from a shared resource, females are expected to consume more than males. This prediction is supported by our study as females gain more weight during breeding than males (paired t test: $t_{59} = 3.37$, $P = 0.00131$). Sexual conflict over consumption may be particularly important in our study species given that it breeds on carcass of small vertebrates that serve as food for both parents and developing larvae (20, 21). However, such conflict may also be important in other systems, such as birds, where the two parents find food for themselves and their offspring within a shared territory.

Although our study provides evidence for female manipulation of male behavior, it leaves an unanswered question as to what mechanisms are responsible. To be effective, such mechanisms must influence the offspring's phenotype, thereby altering the male's behavior in a way that increases the female's or the offspring's fitness at the expense of the male's fitness. Our results reveal that egg size (measured as larval weight at hatching) is not the mechanism responsible for female manipulation of male behavior. Although females reduced egg size in response to the male's presence, we found that males gained more weight when caring for lighter larvae. Thus, the effect of egg size on male weight change was in the opposite direction of the effect due to prenatal conditions. Nevertheless, this result demonstrates that females respond to the presence of the male by adjusting offspring size at hatching, suggesting that females might adjust other maternal effect mechanisms. One potential such mechanism is deposition of maternal

hormones in the eggs (9, 10). There is good evidence that that female birds deposit testosterone into the eggs and that maternal testosterone stimulates nestling begging and growth, although it is debated whether this provides a mechanism for female manipulation of male behavior (10-12). Insects have a different hormonal system from vertebrates, but there is evidence that females deposit juvenile hormones or ecdysone into the eggs (29, 30). Potentially, these maternal hormones might influence larval behavior or development, thereby altering male behavior. Males might also respond to prenatal maternal effect indirectly, by responding to the female's response to the offspring phenotype. We found no evidence for this suggestion, as females did not respond to prenatal maternal effects in our study.

Our study raises a key question: why should males allow themselves to be manipulated by females? After all, if females use maternal effects to enhance their own or their offspring's fitness at the expense of male fitness, we should expect males to be under selection to evolve a counterstrategy to such manipulation (11). In a recent paper, we distinguish between deception, where maternal effects somehow bias the male's behavior away for his own optimum, and incentivization, where maternal effects somehow alter the cost/benefit function of male behavior (9). It is unlikely that deception would be evolutionarily stable because males should simply evolve to ignore manipulating maternal effects. In contrast, incentivization might be evolutionarily stable because maternal effects alter the benefits and/or costs of male behaviors, inducing a change in the male's optimal behavior (9, 31). For example, by depositing hormones into eggs, females might alter the offspring's physiology and growth trajectory, thereby incentivizing males to consume less food for themselves to achieve their cost/benefit optimum.

Conclusion

Until now, most theoretical and empirical work on the resolution of sexual conflict has assumed symmetry of power between males and females with the same mechanisms of conflict resolution applying to both parents (3-5). Our study adds to our understanding of sexual conflict between caring parents by showing that maternal effects provide females with a greater power over their partner's behavior that allow them to manipulate male behavior. Therefore, we urge future work to consider the potential importance of prenatal maternal effects in the resolution of sexual conflict between parents.

Methods

General procedure

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. Beetles were housed individually in clear plastic boxes (124 x 82 x 22 mm) containing moist soil, kept at 21±2 °C under constant lighting and fed small pieces of organic beef twice a week. The beetles were aged 13–24 days post-eclosion at the start of the experiment.

Cross-fostering procedure

We weighed males and females at the beginning of the experiments to record their prebreeding mass. We then placed each pair into a plastic box (110 x 110 x 30mm) with 10mm of moist soil for about 24h to allow all experimental females to be fertilized by a male. We randomly placed either both parents (n = 72) or females only (n = 72) in a larger box (170 x 120 x 60 mm) filled with a 10–20mm layer of soil and provided with a freshly defrosted mouse (21.4–23.7g, supplied from Livefoods

293 Direct Ltd, Sheffield, UK). In the interval between the end of egg laying and the start
294 of hatching (62–63 h after providing the carcass), we moved the parents and their
295 prepared carcass to a new box with fresh soil. Females breeding alone and their
296 prepared carcass were discarded from the rest of the experiment and only boxes
297 with both parents were used as foster parents. At this stage, we counted the
298 number of eggs visible at the bottom of the box as an estimation of clutch size (32).
299 The eggs from both treatments were left to develop in the original box.
300 Larvae hatching from eggs left in the original containers were used to
301 generate experimental foster broods. As soon as possible after their own larvae
302 began to hatch, we provided pairs of breeding beetles with experimental foster
303 broods that differed with respect to whether they hatched from eggs laid in the
304 presence or absence of a male. All experimental broods were comprised of 20
305 larvae from at least 2 different donor pairs that were not the larvae's foster parents.
306 We gave caring parents 20 larvae to match the mean brood size in this species
307 (33). We weighed the larvae before placing them on the carcass as a measure of
308 prenatal maternal investment (34). From the 72 potential receiver pairs, we
309 excluded 5 pairs because their own eggs failed to hatch and 6 other pairs because
310 we did not obtain enough larvae to set up foster broods. Thus, in total, we set up 61
311 experimental pairs. One experimental pair was subsequently excluded from further
312 analyses as one of the parents died during the period of parental care. As we had
313 no prior expectation on effect sizes, we aimed for large and predefined sample size
314 of 30 successful pairs per treatment. The total sample size in the experiment was n
315 = 60 (31 pairs raising broods laid in presence of a male and 29 pairs raising broods
316 laid in absence of a male).
317

Behavioral observations

In order to cover the whole period of parental care (23), we conducted three observations on each pair. We first conducted behavioral observations of parents 1h (±15min) after generating the experimental brood, which is when maternal effects are likely to be most pronounced (32, 35). We then conducted observations after 25h (±15min), which corresponds to the peak in parental care and offspring begging (23). Finally, we conducted observations after 49h (±15min), which is just before larvae become nutritionally independent (23). We used instantaneous sampling, scoring behaviors every 1min for 30min in accordance with established protocols (33). We scored the number of scans each parent spent providing direct care, defined as regurgitation of food to the larvae, manipulation of carrion, or regurgitation of carrion within the crater (36, 37).

Offspring and parental fitness

Parents were left undisturbed until the larvae dispersed from the carcass 8–10 days after pairing, at which point we recorded the number of larvae and weighed the brood. Parents were weighed, placed in individual boxes and checked for survival twice a week as we fed them small pieces of organic beef. We obtained measures of lifespan for n = 57 females (excluding 3 females that escaped from their boxes) and n = 60 males. We placed the dispersed larvae in a box (170 x 120 x 60mm) filled with soil to allow them to pupate and eclose as adults. We then recorded the number and sex of the eclosed offspring. We randomly selected one male and one female offspring from each brood, kept them in individual boxes and fed them twice a week to record their lifespan. Once dead, we measured pronotum width of adult parents and offspring using a Mitutoyo Absolute Digimatic calliper. To minimize

observer bias, all observations and measurements were done blind with respect to the treatment by allocating a two-letters code to each experimental pair.

Statistical analyses

All statistical analyses were conducted using R (38). We used linear models (lm function in stats) except for the behavioral data where we used generalized linear models for zero inflated negative binomial distributions (glmmadmb function in glmmADMB) and for the offspring's adult size where we used linear mixed models (lme function in nlme) given that we measured one male and one female per brood. For survival analyses we used survival models (function survreg and coxph in the package survival). As parametric tests (when an appropriate distribution is available) are statistically more powerful and give more accurate estimates than semiparametric and nonparametric tests (39), we first tried to fit the different available survival distributions to our data and selected the best distribution (log logistic for parents' survival) based on AIC comparison and confirmed graphically that the model fitted our data (see Figure S2). As offspring survival presented an odd distribution (see Figure S1), we used semiparametric Cox proportional Hazard regression models as the effect of the treatment met the assumption of proportional hazards ($\chi^2 = 0.622$, $P = 0.430$). For all analyses, we included the effect of treatment (presence or absence of a male partner before hatching) as well as a small set of pertinent explanatory variables in the full models (see supplementary text). We then applied a backward-stepwise procedure to remove non-significant variables. Statistics of the nonsignificant variables presented in the tables were obtained by sequentially reintroducing each of them into the minimal model (40).

Data Availability

If accepted, the results from this paper will be made available through Dryad

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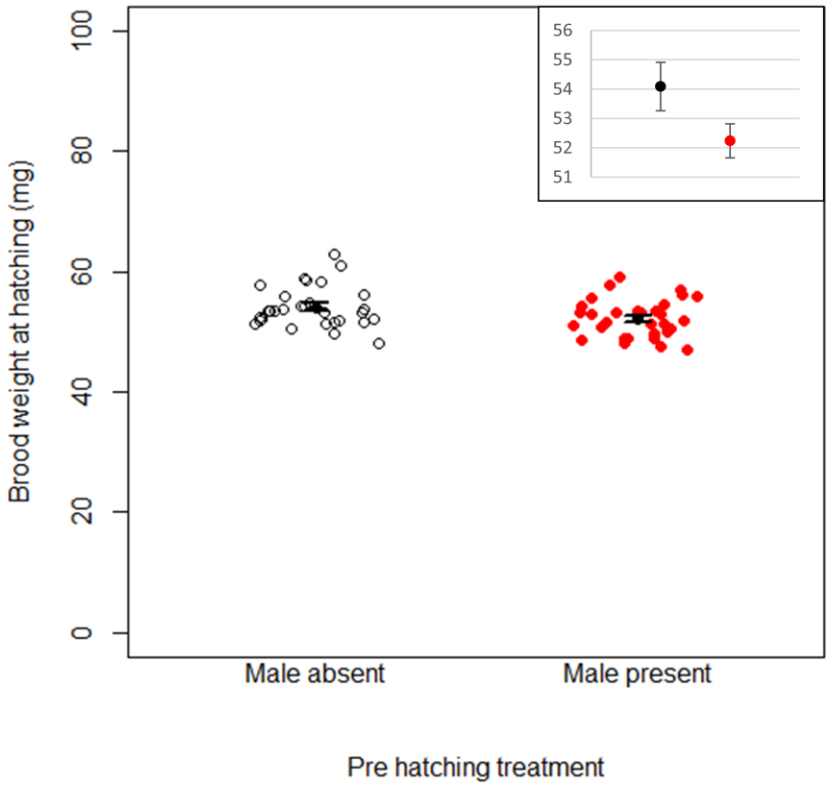
Figures legends

Fig.1. Maternal effect of the presence of the male before hatching on brood weight at hatching. Raw data are shown for brood weight of larvae laid in absence of a male (black open circles) N=30 and brood weight of larvae laid in presence of a male (red filled circles) N=31 as well as associated means ±SE. The inset represents the predicted means±SE from the final model. Experimental broods were all comprised of 20 larvae mixed from different donor pairs of the same pre-hatching treatment that were not the larvae's foster parents. We weighed the 20 larvae together before placing them on the carcass as a measure of prenatal maternal investment.

Fig. 2. Prenatal maternal effects on parental care and weight change. Raw data are shown for the time females and males spent providing direct care 25 hours after hatching **(A)** and females and males weight change from mating to larval dispersal **(B)** when caring for larvae laid in absence of a male (N=29) or in presence of a male (N=31) as well as associated means ±SE. The insets represent the predicted means±SE from the final models. **(C)** Male care 25 hours after hatching was positively associated with male weight change. The dashed lines depict the regression line from the model. Brood weight at hatching and larvae laid in presence of a male have negative effects on male weight change **(D).** The dashed lines depict the regression lines from the final model for each pre-hatching treatment.

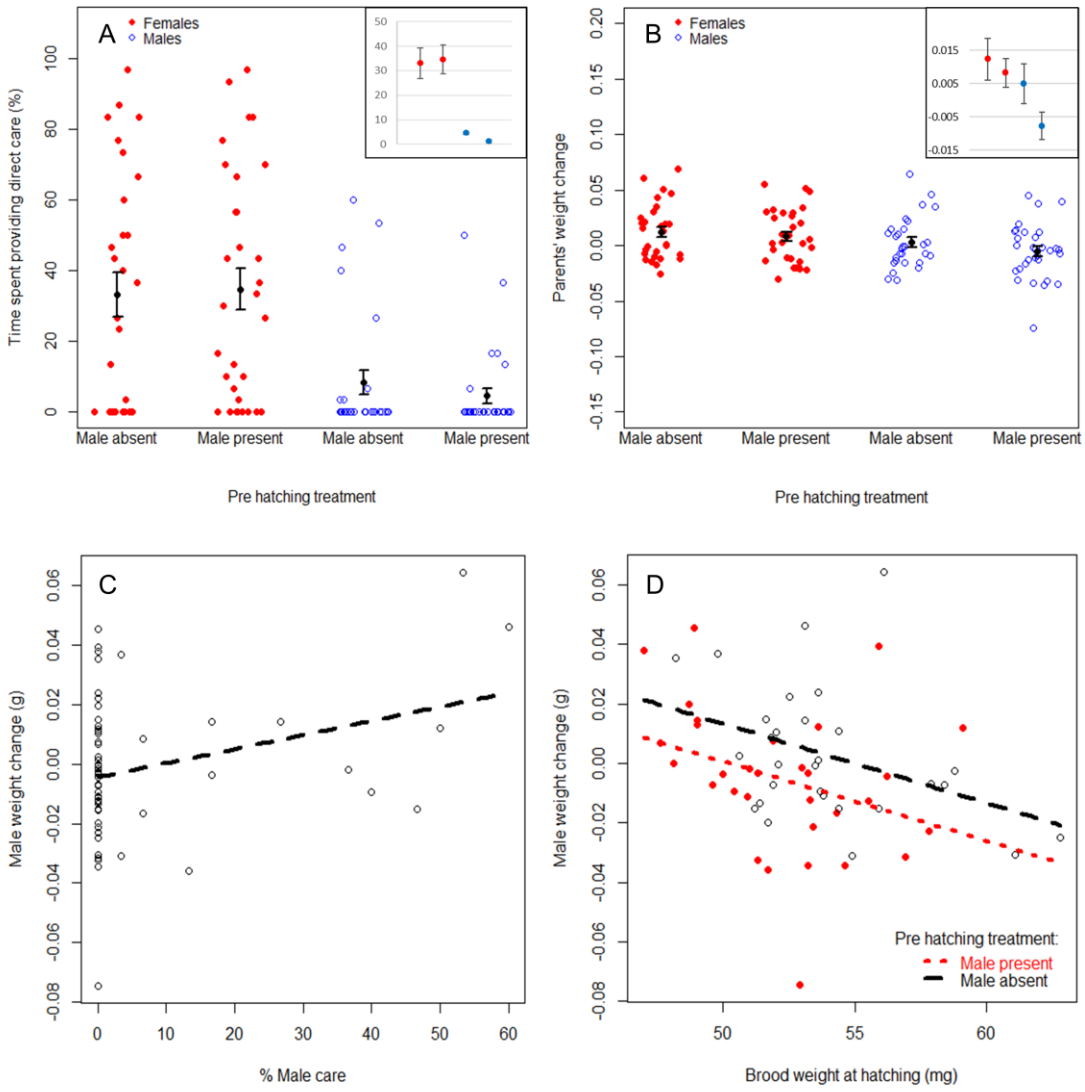
Fig.3. Effect of brood weight at hatching and the prenatal conditions (i.e., presence or absence of a male during laying) on brood weight at dispersal. Raw data are shown (N=60) and the dashed lines depicts the regression lines from the final model for each pre-hatching treatment.



513

514 Fig.1

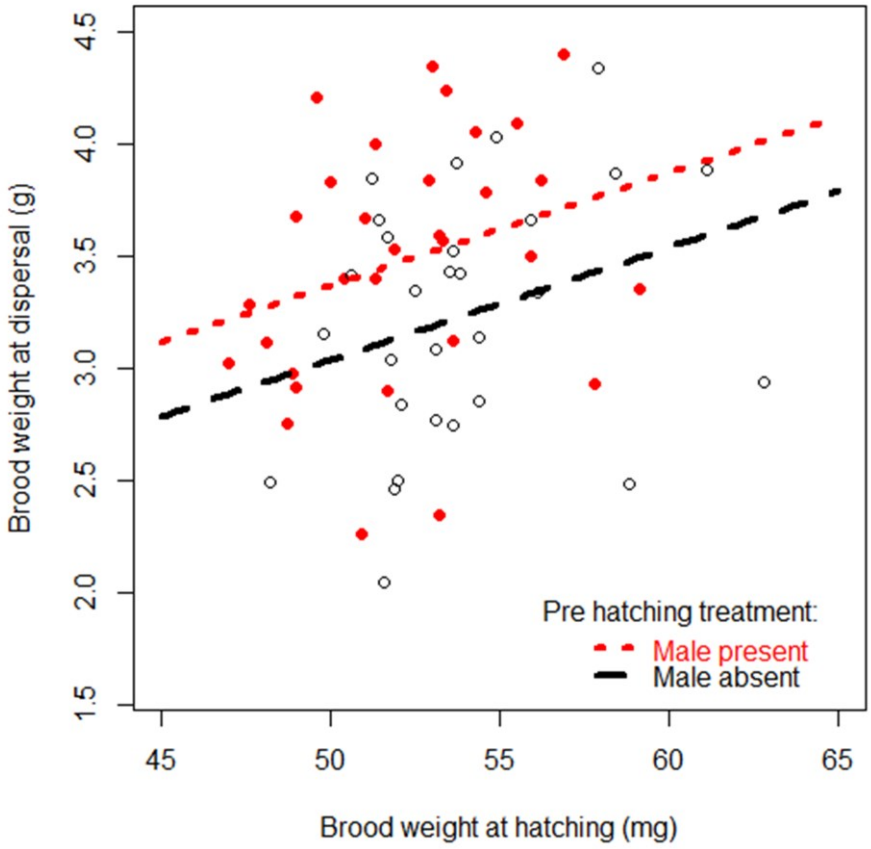
515



516

517 Fig.2

518


Brood weight at dispersal (g)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
45
50
55
60
65
Brood weight at hatching (mg)
Pre hatching treatment:
Male present
Male absent

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520 Fig.3